DECLARATION UNDER 37 CFR § 1.132 OF DR. MICHAEL P. KIRKUP, PH.D.		
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	First Inventor	CARTER et al.
	Art Unit	1625
	Examiner	Dentz, Bernard I.
	Docket #	P07634US01/BAS

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

S I R:

I, Dr. Michael P. Kirkup, Ph.D., declare as follows:

1. I am formerly the Chief Science Officer and Head of Research of J-Star Research, Inc., a company that specialized in custom synthesis and contract chemical research serving the pharmaceutical and biotechnology industries. During my years at J-Star Research, I worked on numerous drug discovery and research matters involving advanced intermediates, designing synthetic routes, designing and implementing process improvements as well as providing resynthesis services. In addition to work I personally performed, I supervised work with many other Ph.D. scientists who were also skilled practitioners in the field of the synthesis of organic chemicals.

2. The present invention relates to compounds which are alternative medicines to Warfarin which has been on the market for over 50 years and whose manufacture is extremely well known. One particular brand of Warfarin is racemic sodium warfarin (also known as Coumadin), a product of Bristol Myers Squibb, and the preparation of this Warfarin is well documented and would be readily known by those skilled in the art.

3. A few years ago, I was asked to prepare a series of Warfarin analogs by New Century Pharmaceuticals, Inc. After reviewing the desired compounds, I was readily able to prepare these compounds using a variety of techniques that were available to me or any other practitioner skilled in this art, and the processes used to prepare these specific compounds were adapted from conventional synthetic reactions, many which had been disclosed in journal references.

4. I am attaching hereto the chemical protocols used by myself and my colleagues in preparing the desired Warfarin analogs, and as indicted in attached chemical reactions, these reflected conventional synthetic methods readily available to skilled practitioners in this field, a number of which related to methods and reactions disclosed in journal articles as reflected in the Appendices. These attachments include: Appendix A, preparation of the AlkylPhenyl series of Warfarin analogs; Appendix B, preparation of the aliphatic series of Warfarin analogs; Appendix C, preparation of 4-(4-hydroxycoumarin-3-yl)-4-(3-carboxyphenyl)-3-butene-2-one sodium salts; and Appendix D, preparation of 4-(4-hydroxycoumarin-3-yl)-6-(3-carboxyphenyl)hexane-2-one sodium salts.

5 In short, my team of chemical researchers was able to prepare the requested Warfarin analogs identified to us by New Century Pharmaceuticals, using tools and techniques that would have been available to other skilled practitioners in this field as well.

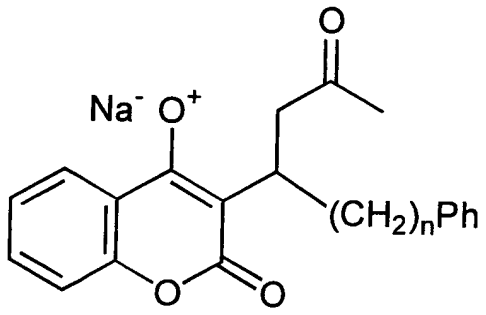
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

8/20/06
Date :

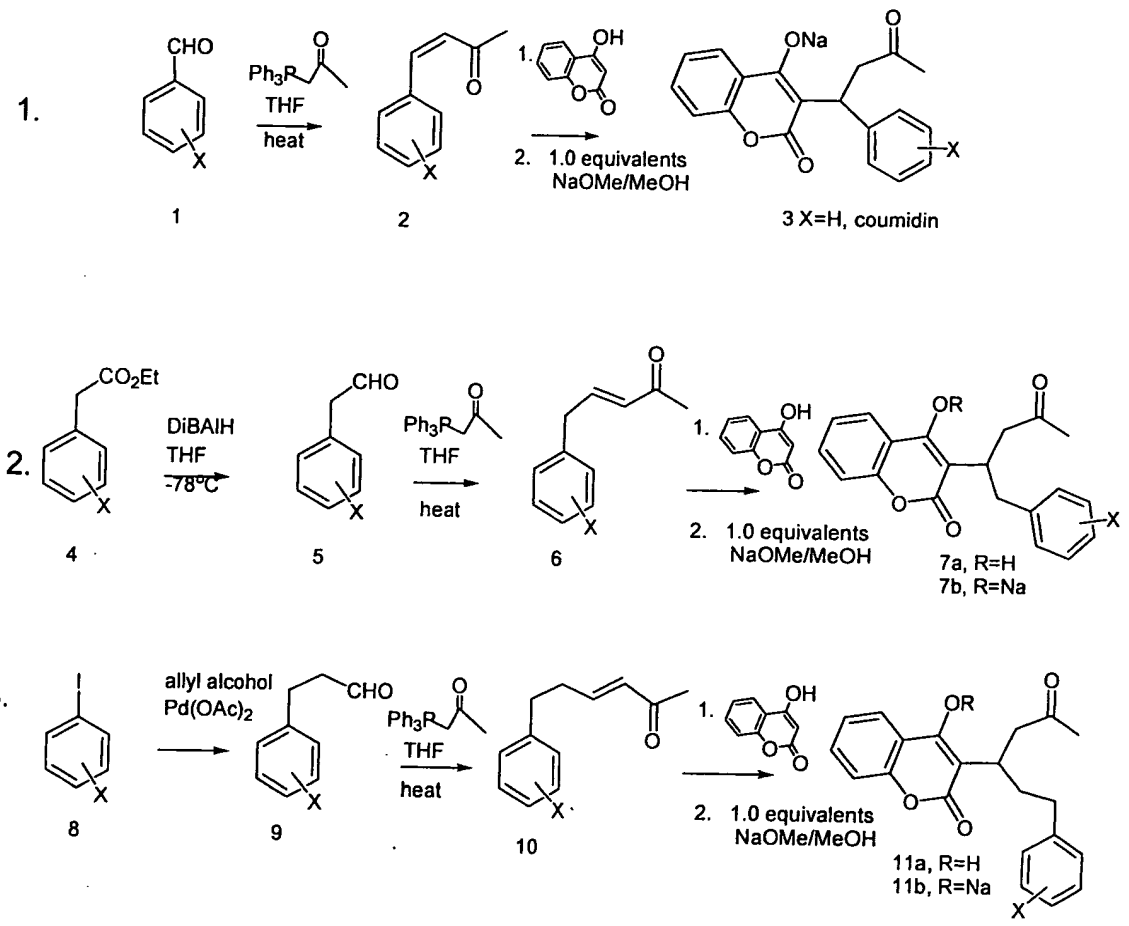
Dr. Michael P. Kirkup, Ph.D.

APPENDIX A

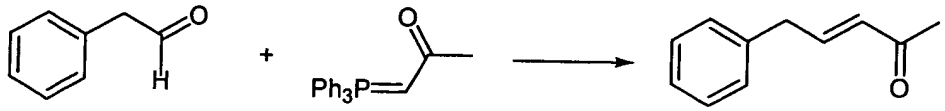
PREPARATION OF WARFARIN ANALOGS – ALKYLPHENYL SERIES



SCHEME 1



Preparation of 1-Phenyl-4-oxopent-2-ene



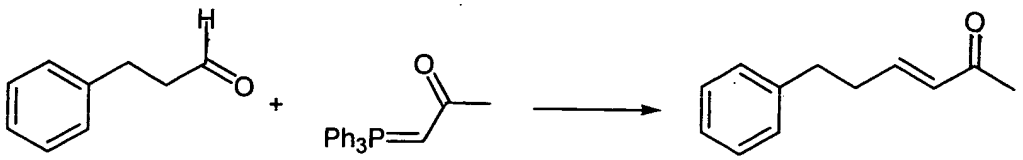
5 **6**

Ref.: Roush, W. et al., *J. Org. Chem.*, **1987**, *52*, 5127-5136.

A solution of compound triphenylphosphorylidene-2-propanone (7 g , 21.88 mmol) dissolved in THF (250 mL) at room temperature. Phenylacetaldehyde **5** in 20 mL THF (2.39 g, 19.89 mmol) was added and the reaction mixture was stirred at 25° C over night. The reaction mixture was concentrated under vacuum to obtain a white solid residue, which was triturated with hexanes. The triphenylphosphine by-product was removed by filtration. Elimination of the hexanes under vacuum afforded 3.8 g of a light amber liquid, which by 1H NMR contains 80 % of the desired compound **6** and 20 % of

the starting material. Purification by flash chromatography using 300 g of silica gel and hexanes ether (7:3) as eluent afforded 2.0 g of **6** contaminated with *ca.* 5 % of the starting material . Yield 63 %.

Preparation of 1-Phenyl-5-oxohex-3-ene



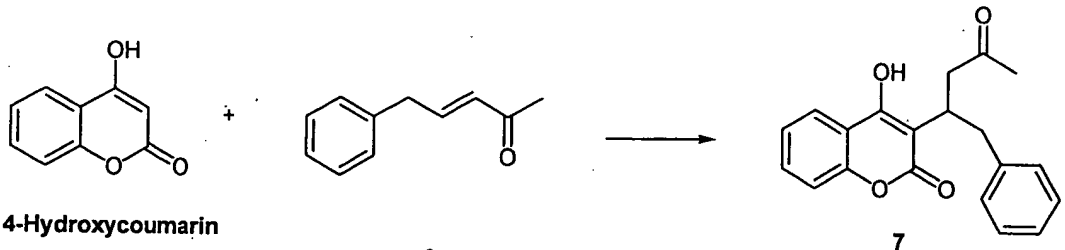
9 **10**

Ref.: Roush, W. et al., *J. Org. Chem.*, **1987**, *52*, 5127-5136.

Triphenylphosphorylidene-2-propanone (1.36 g,, 4.26 mmoles) was dissolved in THF (20 mL) at room temperature under nitrogen and then compound **9** (0.286 g, 2.13 mmoles) dissolved in 7 mL of THF was added. The reaction mixture was vacuum degassed three times and allowed to stir at 23° C with 48 hours. The mixture was concentrated under vacuum to afford a pale beige residue. The triphenylphosphine oxide byproduct was removed by precipitation from hexane followed by filtration. Concentration of the filtrate under reduced pressure afforded a light yellow residue which was purified by flash chromatography [hexanes/ether (9:1)]. The desired product **10** (0.077g) was obtained in 20 % yield.

Note: In another preparation carried out at 0 °C for 14 days at room temperature, the desired compound **10** was obtained in 44 % yield.

Preparation of 3-(1-Benzyl-3-oxobutyl)-4-hydroxycoumarin



4-Hydroxycoumarin **6** **7**

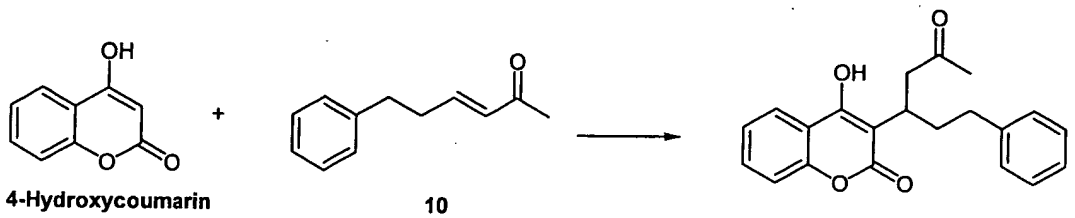
Ref: Ivanov, I., Manolov, I., and Alexandrova, L., *Arch. Pharm. (Weinheim)*, **1990**, 521-522

Manolov, I., and Danchev, ND, *Eur. J. Med. Chem.*, **1995**, *30*, 531-535.

4-Hydroxycoumarin (1.95 g, 12.87 mmol), compound **2** (1.97 g, 12.17 mmol), and benzyl-tri-ethylammonium chloride (0.145 g, 0.64 mmol), were mixed in 45 mL of water and vacuum degassed. The mixture was heated under an atmosphere of nitrogen with stirring at 100 °C for 3.5 hours. The heat was removed and the mixture was allowed to stand without stirring for *ca.* 2 minutes leaving an oily suspension. The hot water was removed by pipette as much as possible. More hot water was added and removed as much as possible. The residue was then dissolved in ethyl acetate, and dried with sodium sulfate. Filtration and concentration under vacuum afforded 4.2 g of crude product **7.** Purification by flash chromatography was carried out using hexanes/ethyl acetate(75:25). Appropriate fractions were combined and solvent was evaporated under vacuum. Pure product 7 (0.65 g) was obtained as a white crystalline solid. Yield 32 %

Note: In another preparation carried out by heating **1** and **2** (1.1 eq.) neat for several hours followed by chromatography of the crude afforded the product in 55% yield.

Preparation of 3-[1-(2-Phenylethyl)-3-oxobutyl]-4-hydroxycoumarin

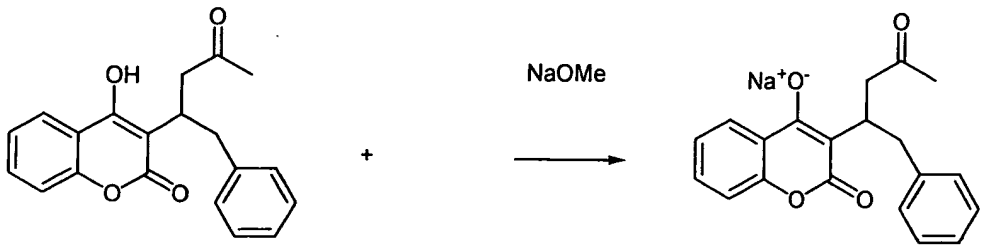


4-Hydroxycoumarin 10 11

Ref:
Ivanov, I., Manolov, I., and Alexandrova, L., *Arch. Pharm. (Weinheim)*, **1990**, 521-522
Manolov, I., and Danchev, ND, *Eur. J. Med. Chem.*, **1995**, *30*, 531-535.

4-Hydroxycoumarin (0.381 g, 2.36 mmol), coumpound 10 (0.373 g, 2.14 mmol), benzyl-tri-ethylammonium chloride (0.0.024 g, 0.107 mmol), and 7.5 mL of water were mixed together and vacuum. The reaction mixture was heated under nitrogen while stirring at 100 °C for 3 hours. Heat was removed and the reaction mixture was allowed to stand without stirring for about 2 minutes and the hot water was removed by pipette. More hot water was added and removed as much as possible. The residue was then dissolved in ethyl acetate and dried with sodium sulfate. Filtration and concentration under vacuum afforded 0.682 g of crude product **11.** Purification by flash chromatography was carried out using hexanes/ethyl acetate(4:1) as eluent and a ratio of 100g of silica gel per 1 g of crude material. Pure **11** was obtained (0.425 gm) as a white crystalline solid. Yield 59 %

Preparation of 3-(1-Benzyl-3-oxobutyl)-4-hydroxycoumarin sodium salt



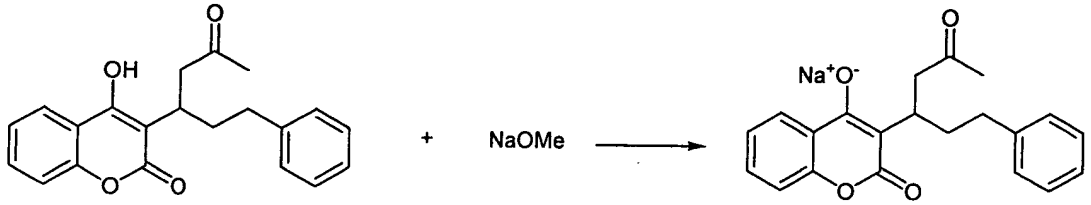
7a **7b**

Ref: Manolov, I., and Danchev, ND, *Eur. J. Med. Chem.*, **1995**, *30*, 531-535.

Fresh sodium methoxide (1 M in methanol) was prepared.

To a round bottom flask containing a solution of compound **7a** (0.511 gm, 1.59 mmol) in methanol (10 mL) was added 15 mL of the previously prepared sodium methoxide solution. The reaction mixture was stirred for 10 min. and concentrated to dryness under vacuum to eliminate the last traces of methanol. Compound **7b** (0.55 g) was obtained as a pale yellow solid.

Preparation of 3-[1-(2-Phenylethyl)-3-oxobutyl]-4-hydroxycoumarin sodium salt



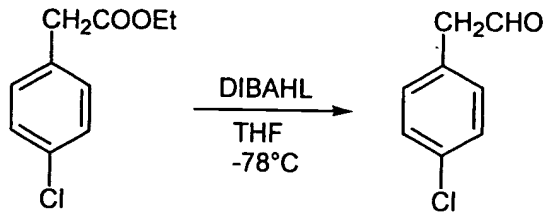
11a **2** **11b**

Ref: Manolov, I., and Danchev, ND, *Eur. J. Med. Chem.*, **1995**, *30*, 531-535.

Fresh sodium methoxide (1 M in methanol) was prepared.

To a round bottom flask containing a solution of compound **11a** (0.420 g, 1.25 mmol) in methanol (10 mL) was added 12.41 mL of the previously prepared sodium methoxide solution. The reaction mixture was stirred for 10 min. and concentrated to dryness under vacuum to eliminate the last traces of methanol. Compound **11b** (0.48 g) was obtained as a pale yellow solid.

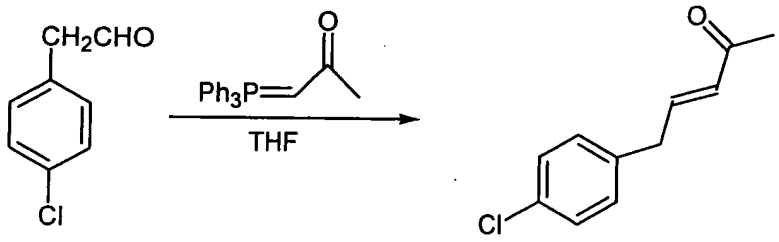
4-Hydroxy-3-[1-(4-chloro-benzyl)-3-oxo-butyl]-coumarin sodium salt



4 **5**

The ester **4** (12.0g, 0.06mol) was dissolved in THF (105.0mL) and cooled to -78°C in a dry-ice bath. DIBAHL (52.3mL, 0.078mol, 1.3eq) in toluene was added dropwise from a addition funnel over 2hr under a nitrogen atmosphere. The reaction mixture was stirred for 1hr and quenched by addition of Roschelle's solution at -78°C. The reaction mixture was warmed to rt and stirred for 1hr until gel-like precipitate was observed.The precipitate was removed by filtration through celite and washed with THF (~700mL) several times. The filtrate was concentrated by evaporation under reduced pressure. The crude colorless aldehyde **5** (11.0g) was obtained as an oil and used in the next reaction without purification. Rf 0.35 (Hex/AcOEt=5/1)

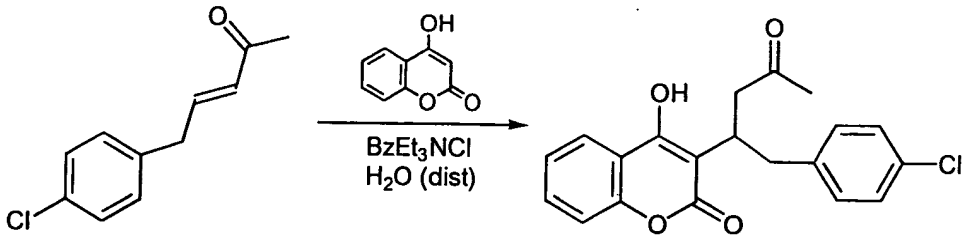
Preparation of 5-(4-chlorophenyl)- 3-penten-2-one



5 **6**

Aldehyde **5** (4.48g, 0.025mol) was dissolved in THF (100mL) and cooled to 0°C in a ice-water bath. A solution of 1-triphenylphosphoranylidene-2-propanone (8.6g, 0.027mol, 1.1eq) in of THF (200mL) was added dropwise over 1hr from an addition funnel at 0°C under N_2 atmosphere. After 30min at 0°C, the reaction mixture was warmed to rt and stirred for 16hr. The solvent was removed by evaporation under vacuum. The residual solid was transferred into a glass filter and washed with Hex/AcOEt (3:1) several times. The filtrate was concentrated by evaporation under reduced pressure. The crude yellow mixture was purified by flash column chromatography (SiO_2, 230-400mesh, Hex/AcOEt=8/1-5/1) to obtain **6** as a pale yellow oil (1.1g, 23% over 2 steps). Rf 0.36 (Hex/AcOEt=3/1)

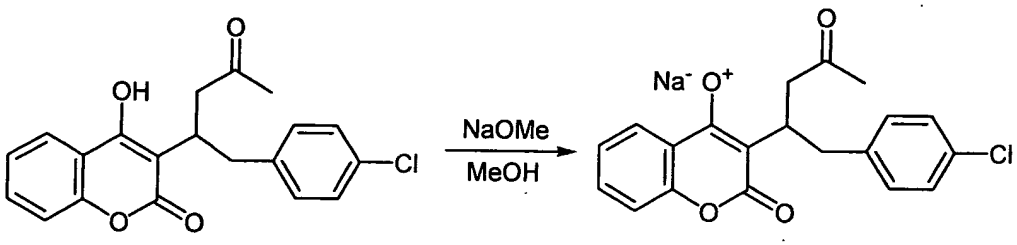
Preparation of 3-(1-(4-chlorobenzyl)-3-oxobutyl)-4-hydroxycoumarin



3 4

Enone **6** (1.55g, 8.0mmol) and 4-hydroxycoumarin (1.55g, 9.58mmol, 1.2eq) were put into a flask. Water (dist, 23.0mL) was added, followed by the addition of 91.0mg of $BzEt_3NCl$ (0.40mmol, 0.05eq) as a solid. The mixture was degassed and purged with nitrogen. The reaction mixture was heated under reflux for 18hr. Some starting material remained and additional 4-hydroxycoumarin (0.75g, 4.62mmol, 0.57eq) was added. Then the mixture was heated under reflux for 4.5hr and again 4-hydroxycoumarin was added (0.75g, 4.62mmol, 0.57eq). After reflux for 2hr, the mixture was cooled to room temperature. The yellow oily suspension was extracted with ethyl acetate (~250mL), washed with sat NaClaq (100 mL), and concentrated under vacuum. The crude yellow mixture was purified by flash chromatography (SiO_2, 230-400 mesh, 130g, Hex/AcOEt 5/1-3/1-1/2, crude was pre-adsorbed on silica gel (10 gm) to apply.) The product **7a** was obtained as a semi-pure yellow solid and was again purified by flash column chromatography (SiO_2, 230-400mesh, 100g, Hex/AcOEt=2/1) to obtain 0.284g of a pale yellow solid (cy 22%).

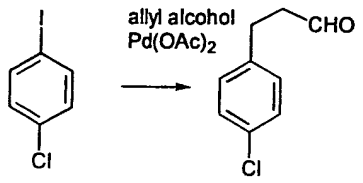
Preparation of 3-(1-(4-chlorobenzyl-3-oxobutyl)-4-hydroxycoumarin sodium salt



7a 7b

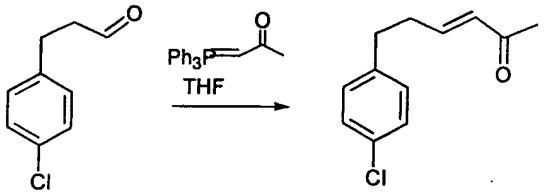
Compound **7a** (0.637g, 1.78mmol) was dissolved in of MeOH (60.0mL, anhydrous) with slight heating. Freshly prepared sodium methoxide in MeOH (0.13M, 13mL, 1.73mmol, 0.97eq) was added at rt. After stirring for 15 min., the solvent was removed by evaporation under reduced pressure. The resulting yellow solid was transferred into a glass filter and washed with MTBE several times. The solid was taken into a flask and dissolved in 1.5mL of H_2O (dist). The water was removed by evaporation under reduced pressure. The product **7b** was obtained as a pale yellow solid (0.694g) and was dried under vacuum. Rf 0.32 (Hex/AcOEt, 1:1).

Preparation of 3-(4-chlorophenyl)propionaldehyde



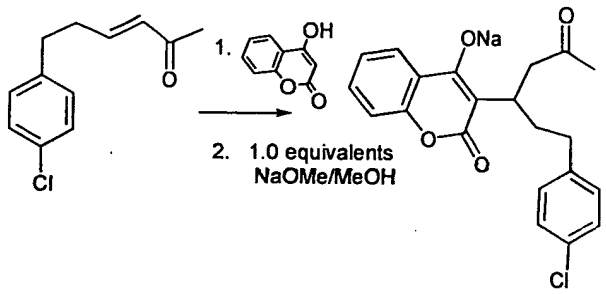
To a 1 liter three neck flask containing DMF (500 mL) which was vacuum purged with nitrogen, was added 4-chloroiodobenzene (24 g, 0.1 mole), allyl alcohol (9.2 g, 0.15 mole), palladium acetate (0.450 gm, 0.002 moles), tetrabutyl ammonium chloride (28 g, 0.10 moles) and solid sodium bicarbonate. The mixture was stirred vigorously under nitrogen overnight at room temperature. The reaction was dikuted with 1.5 liters of water and product was extracted with 2x's 1 liter of 10 % ethylacetate/hexane. The organic layers were combined and solvent was evaporated under vacuum to give 16.1 gms of 3(4-chlorophenyl)propionaldehyde (0.095 moles, 96% yield) which was used in the next step without further purification.

Preparation of 6-(4-chlorophenyl) hex-3-ene-2-one



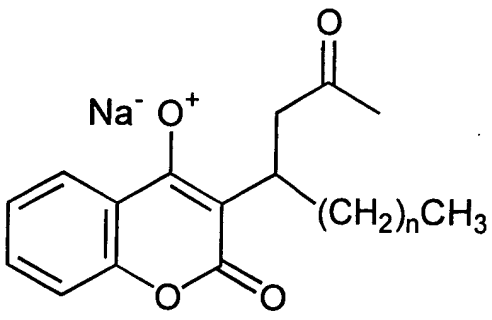
To a 500 mL three neck round bottom flask was added 3(4-chlorophenyl)propionaldehyde from the previous reaction (16.1 gm, 0.09 moles), THF (300 mL) and 1-triphenylphosphoranylidene-2-propanone (38 gm, 0.12 moles). The mixture was heated to reflux under nitrogen for 3 hours. The heat was removed and the reaction was stirred at room temperature under nitrogen overnight. The reaction mixture was diluted with hexane (2 L), solid triphenylphosphine oxide was removed by filtration and the filtrate was concentrated to a semisolid. This residue was triturated with hexane (100 mL) and additional byproduct was removed by filtration. Solvent was removed by evaporation under vacuum and the oily residue was purified by silica gel chromatography. The appropriate fractions were combined and solvent was evaporated to give pure 6-(4-chlorophenyl) hex-3-ene-2-one (12.3 gm, 0.058 moles, 65%).

Preparation of 6-(4-chlorophenyl)-4-(4-hydroxycoumarin-3-yl)-2-hexanone sodium salt



APPENDIX B

PREPARATION OF WARFARIN ANALOGS – ALIPHATIC SERIES



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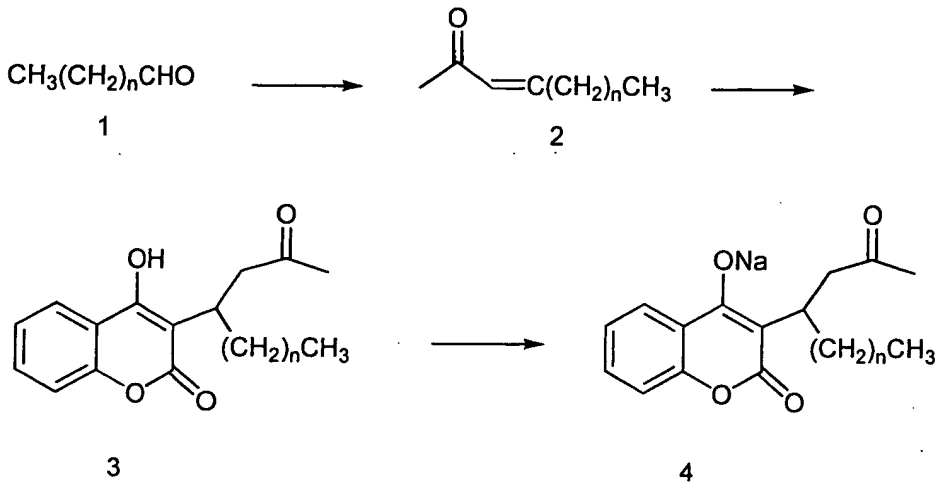


Experimental Section

Typical preparation of aliphatic enones (2)

Valeraldehyde (**1**, n=3, 2.0g, 23.2mmol) was dissolved in 45mL of THF. Triphenylphosphoranylidene-2-propanone (8.13g, 25.5mmol, 1.1eq) was added as solid. The reaction mixture was heated under reflux for 17.5hr. After cooling to room temperature, the solvent was removed by evaporation under reduced pressure. The residual solid was suspended in Hex/AcOEt (5/1) and passed through short silica gel bed (~20g) and eluted with Hex/AcOEt (5/1) several times (~300mL). The eluent was concentrated by evaporation under reduced pressure. The semi-purified product **2** was purified by flash column chromatography (SiO_2, 230-400 mesh, 100g, Hex/AcOEt=10/1) to obtain 2.61g of colorless oil (cy 89%). See table 1 for yield and other data for other members of the series.

Note: In subsequent runs it was found that most of the triphenylphosphine by-product and be removed by addition of hexane to the concentrated crude reaction mixture.

Table 1

	cy (%)	Rf	Hex/AcOEt
n=3	89	0.42	10/1
4	96	0.54	5/1
5	29	0.61	5/1
7	40	0.55	5/1

Typical Michael addition of 4-Hydroxycoumarin to aliphatic enones

To 15mL of distilled water was added the enone derived from nonaldehyde **2** (n=7, 5.48mmol) and 0.98g of 4-hydroxycoumarin (6.03mmol, 1.1eq), followed by addition of 62.4mg of $BzEt_3NCl$ (0.274mmol, 0.05eq) as a powder. The whole was degassed under vacuum and purged in a nitrogen stream. The reaction mixture then was heated under reflux for 3 hr under a nitrogen atmosphere. The resulting oily suspension was extracted into ethyl acetate (2x20 mL) and the organic layers were combined, washed with 75mL of sat brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The residual oil was dissolved in hexane/ethyl acetate (1/1) and passed through short bed of silica gel (~50g). The silica gel was washed several times with hexane/ethyl acetate (1/1). The solvent was removed by vacuum distillation. The oily residue was purified by flash column chromatography (SiO_2, 230-400 mesh, 60g, Hex/AcOEt=10/1-1/1) to obtain 1.37g of **3** as a colorless oil (cy 73%). See Table 2 for results on other members of the series.

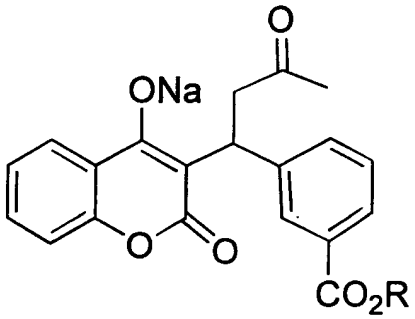
	cy (%)	Rf	Hex/AcOEt
n=3	46	0.39	1/2
4	59	0.50	1/1
5	71	0.33	5/1
7	73	0.59	1/1

Typical preparation of warfarin analog sodium salts

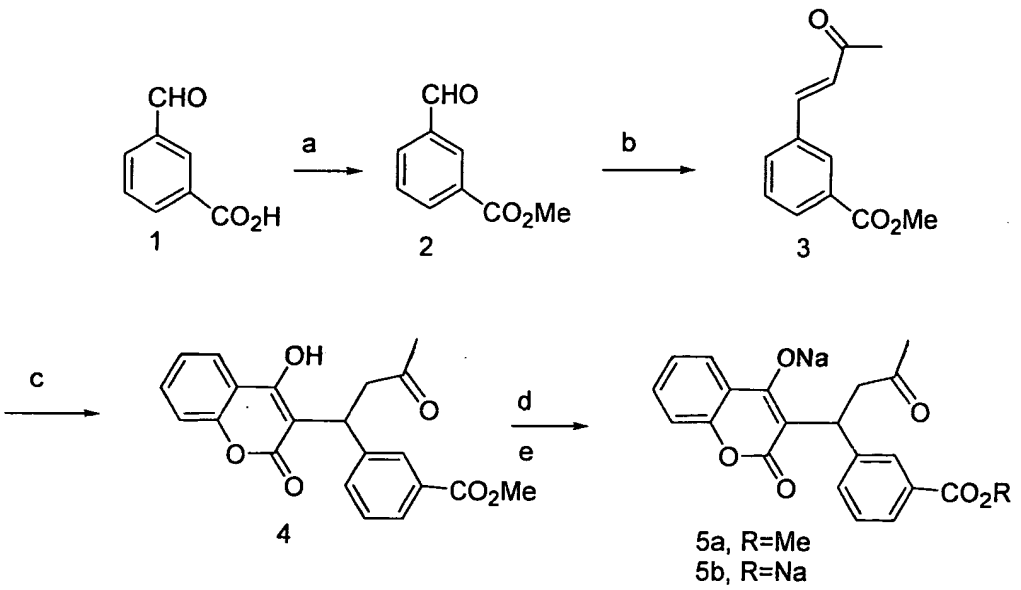
To a solution of **3** (n=4, 7.84mmol) in of MeOH (30mL, anhydrous) was added a solution of freshly prepared NaOMe (175mg of Na metal and 16mL of anhydrous MeOH). The reaction mixture was stirred for 10min and the solvent was removed under reduced pressure. The residual solid was transferred into a glass filter and washed with MTBE several times. The pale yellow solid was taken into a flask and dissolved in 3mL of distilled water. The water was removed under reduced pressure and the solid was dried in a vacuum oven at 45°C. To give product 4 as a pale yellow solid (2.37g, cy 96%).

APPENDIX C

PREPARATION OF WARFARIN ANALOGS – 4-4(HYDROXYCOUMARIN-3-YL)-4-(3-CARBOXYPHENYL)-3-BUTENE-2-ONE SODIUM SALTS



R= Me, Na



a) MeI, K_2CO_3, DMF b) triphenylphosphorylidene-2-propanone, THF, reflux. c) 4-hydroxycoumarin (neat), 150°C, 3 hr. d) 1 eq. NaOMe, MeOH. e) I eq. NaOH, MeOH

Preparation of 3-carbomethoxybenzaldehyde (2)

To a stirred solution of 3-carboxybenzaldehyde (**1**, 13.5 gm, 90 mmoles) in DMF (60 mL) was added potassium carbonate (14.94 gm, 110 mmoles) and methyl iodide (5.6 mL, 90 mmoles). The reaction was kept at room temperature for one hour. The reaction was diluted with 600 mL of 10% EtOAc/hexane and the salts were removed by filtration. The filtrate was washed with 5% Na_2SO_3 (200 mL) and brine (2x 100 mL). The organic layer was dried (Na_2SO_4) and solvent was evaporated under vacuum to give **2** as a white solid (11.5 gms, 70.2 mmoles, 78% yield).

Preparation of 4-(3-carbomethoxyphenyl)-3-butene-2-one (3)

To 3-Carbomethoxybenzaldehyde (**2**, 11.5 gm, 70 mmoles) in THF (220 mL) was added triphenylphosphorilidene-2-propanone and the reaction mixture was heated at reflux for 3 hours under nitrogen. Heat was removed and the reaction was stirred atr room temoerature for 18 hrs.under nitrogen. The mixture was diluted with 1.5 L of hexane and insoluble material was removed by filtration. The filtrate was concentrated to dryness to give 17 grams of **3** as a white solid (contains triphenylphosphine oxide). This material was suspended in 40 mL of 10% EtOAc/hexane and applied to a pad of silica gel. Product was eluted wit 10% EtOAc/hexane. Appropriate fractions were combined and solvent was evaporated under vacuum leaving 12.86 gms (63 mmoles, 90% yield) of pure eneone **3**.

Preparation of 4-(4-hydroxycoumarin-3-yl)-4-(3-carbomethoxyphenyl)-3-butene-2-one (4)

4-(3-Carbomethoxyphenyl)-3-butene-2-one (20.4 gms, 100 mmoles) and 4-hydroxycoumarin (15.6 gms, 95 mmoles) were placed in a flask and heated neat with stirring at 150°C under nitrogen for three hours. The reaction mixture is cooled and dissolved in 50 ml of EtOAc. Silica gel (40 gms) was added and the solvent was evaporated under vacuum. The preadsorbed material was placed on top of a column of flash grade silica gel (400 gms) and the less polar impurities were eluted with hexane (2 L). The desired product was eluted with 50% EtOAc/hexane (2L) and solvent was evaporated under vacuum to leave a solid which was taken up into 300 mL of EtOAc and slowly diluted with hexane. The cloudy suspension was aged for several hours and the white precipitate was collected by suction filtration. The filter cake was dried under vacuum to give 25 gms (65 mmoles, 72% yield) of pure material .

Preparation of 4-(4-hydroxycoumarin-3-yl)-4-(3-carbomethoxyphenyl)-3-butene-2-one monosodium salt (5a)

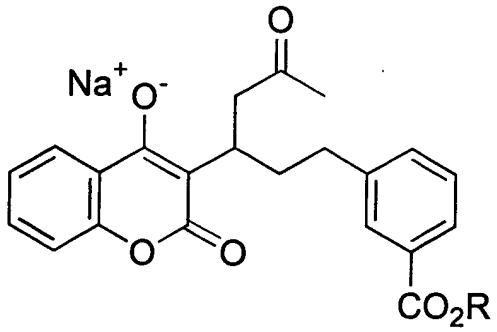
Product from above can be dissolved in MeOH and treated with 1.0 Eq. of NaOMe in MeOH at 10°C. Upon evaporation of solvent under vacuum a quantitative yield of the monosodium salt **5a** is obtained.

Preparation of 4-(4-hydroxycoumarin-3-yl)-4-(3-carboxyphenyl)-3-butene-2-one disodium salt (5b)

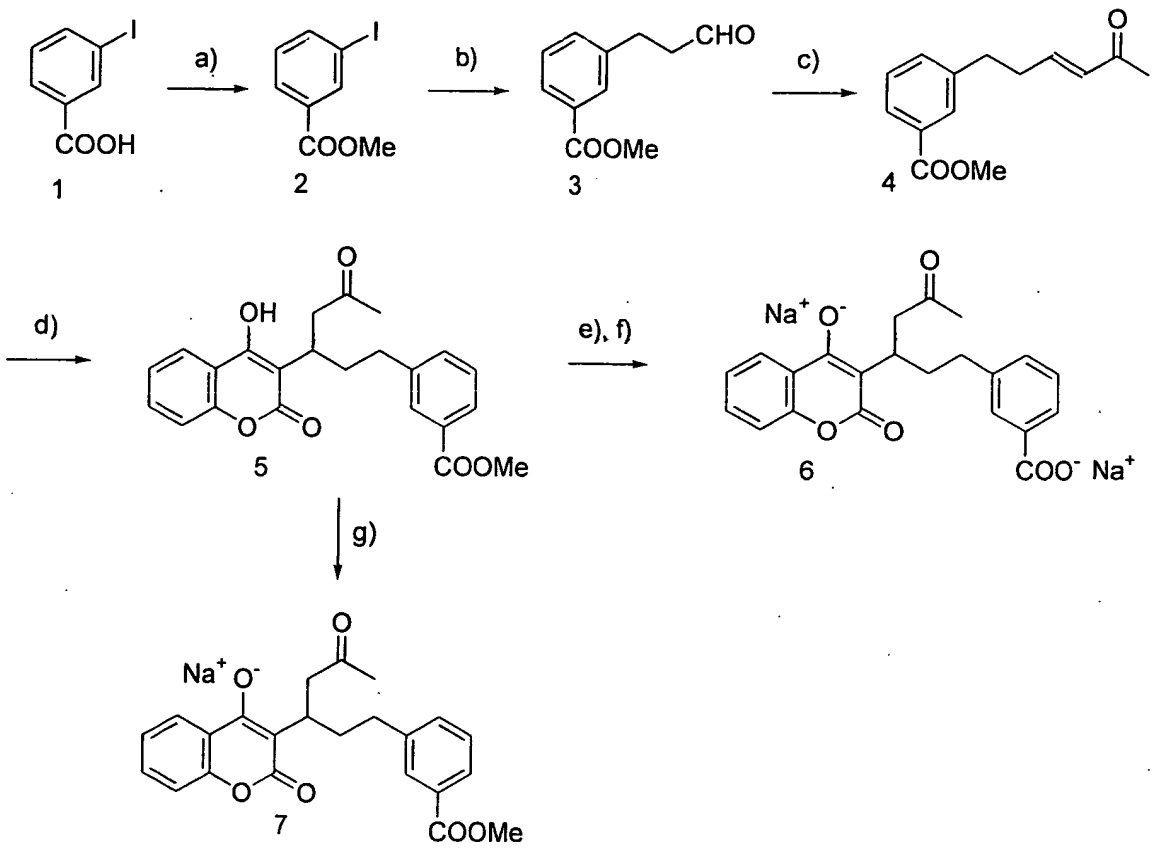
Product from above can be dissolved in MeOH and treated with 1.0 Eq. of NaOMe in MeOH at 20°C for 10 min. Upon evaporation of solvent under vacuum a quantitative yield of the disodium salt **5b** is obtained.

APPENDIX D

PREPARATION OF WARFARIN ANALOGS – 4-4(HYDROXYCOUMARIN-3-YL)-6-(3-CARBOXYPHENYL)HEXANE-2-ONE SODIUM SALTS



R= Me, Na



a) MeI, K_2CO_3, DMF, rt. b) Allyl alcohol, $Pd(OAc)_2$, $NaHCO_3$, Bu_4NCl, DMF, rt. c) 1-Triphenylphosphoranylidene-2-propanone, THF, reflux, 78%. d) 4-Hydroxycoumarin, 150°C, 60%. e) NaOMe, MeOH, H_2O, 84%. f) NaOMe, MeOH, quant. g) NaOMe, MeOH, 84%.

Preparation of methyl 3-iodobenzoate (2)

To a solution of 15.3g of 3-iodobenzoic acid (0.061mol) in 60mL of DMF was added 10.1g of K_2CO_3 (0.073mol, 1.2eq) as a powder, followed by addition of 4.2mL of MeI (0.068mol, 1.1eq) at rt. The reaction mixture was stirred vigorously for 1.5hr at rt. Then the whole was diluted with 400mL of ethyl acetate. The precipitate was removed by filtration. The filtrate was diluted with ethyl acetate (800mL) and hexane (150 mL) The organic layer was washed with 90mL of 5% $Na_2S_2O_3$aq, 90mL of H_2O 5 times, 120mL of sat NaCl (aq.), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure to obtain 15.8 g of pale yellow oil. (Rf 0.87, Hex/AcOEt=5/1). The crude mixture was used for the next step without purification.

Preparation of 3-(3-carbomethoxyphenyl)propionaldehyde (3)

To a solution of 36.9g of 3-iodobenzoic acid methyl ester (0.141mol) in 300mL of DMF was added 12.2g of allyl alcohol (0.211mol, 1.5eq), 27.2g of $NaHCO_3$ (0.324mol, 2.3eq), 39.2g of Bu_4NCl (0.141mol, 1eq), and 0.63g of $Pd(OAc)_2$ (2.81mmol, 0.02eq) under a nitrogen atmosphere. DMF (70ml) was used to rinse. The reaction mixture was stirred

mechanically for 22 hr. at room temperature under N_2. The whole was diluted with 600mL of Hex/AcOEt (1/1). The precipitate was removed by filtration through Celite and washed with additional 600mL of Hex/AcOEt (1/1). The filtrate was transferred into a separatory funnel, washed with H_2O (2 x 450 mL), sat (aq.) NaCl (600mL), dried over Na_2SO_4, and filtered. The filtrate was passed through a short silica gel bed eluting with Hex/EtOAc (1/1). The Filtrate was concentrated under reduced pressure to obtain 24.4g of brown oil. (Rf 0.16, Hex/AcOEt=10/1). The crude mixture was used for the next step without further purification.

Preparation of 6-(3-carbomethoxyphenyl) hex-3-ene-2-one (4)

To a solution of 25.6g of 3-(3-carbomethoxyphenyl)propionaldehyde (0.133mol) in 385mL of THF was added 3-triphenylphosphoranylidene-2-propanone (63.6g, 1.5eq). The reaction mixture was heated under reflux for 4.5hr. After cooling to r.t., the solvent was removed under reduced pressure. The pale yellow crude oil was dissolved in an small amount of ethylacetate/ hexane (1:1), dried on a small amount of silica gel and applied on silica gel column (230-400mesh, 510g, Hex:AcOEt=10:1-5:1) to obtain 23.8g of pale yellow oil (cy 77%). (Rf 0.25, Hex/AcOEt=5/1).

Preparation of 4-(4-hydroxycoumarin-3-yl)-6-(3-carbomethoxyphenyl)hexane-2-one (5)

6-(3-carbomethoxyphenyl) hex-3-ene-2-one (19.0g, 0.082mol, 1.2eq) and 4-hydroxycoumarin (11.0g, 0.068mol) were mixed neat in a round bottom flask. After purging with nitrogen, the yellow heterogeneous reaction mixture was heated at 150°C for 2.5hr. (the mixture became an orange homogeneous liquid.) After cooling to room temperature, the crude mixture was solidified using seed and triturated in hexane. The resulting yellow gummy paste was suspended in 200mL of Hex/AcOEt (5/2) to remove unreacted enone. (the yellow gummy paste became a firm solid.). The solid was isolated by filtration, washed with of Hex/EtOAc (5/1, 2x150mL) and dried (Na_2SO_4) to obtain 16.1g of off white solid (cy 60%). (HPLC >99%). (Rf 0.29, Hex/AcOEt=1/1).

Preparation of 4-(4-hydroxycoumarin-3-yl)-6-(3-carboxphenyl)hexane-2-one di-sodium salt (6)

To a solution of 4-(4-hydroxycoumarin-3-yl)-6-(3-carbomethoxyphenyl)hexane-2-one (12.0g, 0.03mol) in 120mL of MeOH was added 24mL of H_2O (20%, v/v), followed by slow addition of 8.2g of NaOMe (0.15mol, 5eq) as powder (exothermic). Reaction mixture was heated at 50°C for 3hr and cooled to rt. The crude mixture was acidified with 10% HCl (aq) (65mL) and diluted with EtOAc (480 mL). The organic layer was separated, washed with H_2O (2 x 50 mL),sat NaClaq (2 x 70mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure. The resulting solid was transferred into a glass filter, washed with water several times and with MTBE 2 times. Impurities were removed by washing with acetonitrile and then Hex/EtOAc (1/1) several times, and dried under vacuum affording 9.6g of an off white solid (cy 84%). (HPLC >99%). (Rf 0.09, CH_2Cl_2/MeOH=10/1). To a suspension of the 4-hydroxycoumarin

conjugate carboxylic acid (9.33g, 0.0245mol) in MeOH (37mL) was added 2.63g of NaOMe (0.0488mol, 1.99eq) as powder. The mixture became a homogeneous yellow solution.. The reaction mixture was stirred for 15 min. at room temperature, and then the solvent was removed under reduced pressure. The resulting orange oil was solidified by addition of hexane(100 mL) and evaporation under reduced pressure several times. After drying, a pale yellow solid was obtained (quant). (HPLC >99%)

Preparation of 4-(4-hydroxycoumarin-3-yl)-6-(3-carboxphenyl)hexane-2-one mono-sodium salt (7)

The coumarin conjugate methyl ester **(5)** (5.0g, 0.0127mol) was suspended in MeOH (25mL). Sodium methoxide (678.0mg of 0.0125mol, 0.99eq) was added as a powder. The reaction mixture was stirred for 15min at rt. (After the addition, the mixture became a homogeneous yellow solution.) The solvent was removed under reduced pressure. The crude amorphous material was suspended in distilled water. A small amount of an insoluble yellow semisolid was removed from water layer by filtration. The water layer was concentrated under reduced pressure. During concentration, the yellow semisolid was observed and repeatedly removed by filtration. The resulting orange oil was suspended in hexane (20 mL) and concentrated under reduced pressure several times to obtain 4.4g of yellow solid (cy 84%). (HPLC 99%).